ing the growth and maturation of oocytes. Previously we found an inhibiting effect of noradrenaline, dopamine and euphylline in relation to the levels of labelled uridine and leucine uptake into growing oocytes of the sea urchin, *S. nudus*[9,10]. Evidently inhibition of oocyte growth under the influence of catecholamines is connected with a depression of RNA and protein biosynthesis. Hypothetically the above data may by explained by catecholamine acting on the cell membranes and activating adenyl cyclase, which promotes the formation of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) from ATP. At present this mechanism has been described both for mammals and invertebrates[11, 12]. The effects of cyclic AMP, dibutyryl cyclic AMP and inhibitors of cyclic nucleotide phosphodiesterase are compensated or complete inhibition of oocyte maturation in mice and rats[13–15]. It has also been found that cAMP participates in the regulation of some metabolic processes in sea urchin eggs[16,17]. Proceeding from the data obtained we have come to the conclusion that the monoaminergic system does participate in the regulation of oocyte growth and maturation in sea urchins. As no adrenergic nerve fibers have been found in sea urchin gonads[18] it might be proposed that catecholamines control the functions of sea urchin sexual glands by neurohumoral means.

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Plasma transcortin concentration in thyroidectomized chick embryos

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Summary. Concentrations of transcortin binding sites and apparent dissociation constants for corticosterone have been measured in the blood of control and thyroidectomized chick embryos. The levels of corticosterone binding are quantitatively similar in normal and thyroidectomized embryos and vary in parallel during development.

In the blood of the chick embryo a protein has been detected that binds progesterone and corticosteroids with high affinity and limited capacity[1]. This embryonic transcortin-type protein, homologous with the adult chick transcortin, was found to be synthesized by the liver[2,3]. Transcortin concentration in embryonic chicken plasma increased up to day 15 of incubation and then sharply decreased until day 20[1]. The evident consequence of this drop in C_{21}-steroid binding protein is an increase of unbound C_{21} steroids in blood during the last days of incubation. Thus free steroids, the active form of hormones, are available to receptor sites in steroid target cells[4]. More recently transcortin contents of embryonic plasma have been measured in partially decapitated (i.e. hypophysectomized) chick embryos[5]. A specific effect of partial decapitation, i.e. the inhibition of the drop in transcortin level, was observed after day 15 of incubation. Thus the transcortin level in the blood appears to be controlled at the pituitary level but it is not known whether this is a direct effect of the hypophysis or if other organs and indirect pathways are involved. The thyroid, which is affected by decapitation[6,7] could be a candidate for transcortin regulation. The aim of the present study was to analyze in ovo the effects of thyroid on transcortin activity in the plasma of the chick embryo. We have devised a microsurgical procedure by which the thyroid primordium is removed at day 5 or 6 of incubation.

Material and methods. Outbred white Leghorn chick embryos were incubated at 38 °C in a humid environment. Experimental embryos were obtained through a microsurgical procedure. Eggs at 5 or 6 days of incubation were opened at the blunt pole above the air chamber. Shell membrane, chorion and amnion were torn open. The torn amnion was used as a cord one end of which remained connected to the umbilicus, while the other was pulled over to the egg shell where it adhered. In order to extend the neck, the head of the embryo was pulled with a hairloop in the opposite direction to that of the umbilical cord (fig. 1). After skin incision, the median bilobed thyroid primordium (5-day-old embryos) or the 2 separate thyroid glands (6-day-old embryos) were dissected with a small hairloop and

Corticosterone binding capacities in the plasma of chick embryos

Days of incubation	Number of determinations*	Corticosterone binding capacity (10^{-9} M)
13, Control	5	164±54**
13, Thyroidectomy	3	188±97; NS***
17, Control	3	141±17
17, Thyroidectomy	4	178±111; NS
20, Control	9	50±28
20, Thyroidectomy	7	59±29; NS

* Each determination was made on a sample of blood obtained from one embryo. ** Mean± SD. *** Not significantly different (NS) from the preceding value ($p > 0.05$) (Student's t-test).

extirpated with forceps. The amnion was cut away and the egg closed with scotch tape and put back in the incubator until sacrifice. Operated embryos appeared normal during most of the incubation time. It was only in the last third of the incubation period that they exhibited a marked decrease in weight; besides that, they did not retract their yolk sac and finally they died before hatching. Up to now, we have not succeeded in obtaining thyroidectomized embryos older than 20 days of incubation.

Isotope-labelled (1,2-^{3}H) corticosterone (New England Nuclear, 82 Ci/mmole) was shown to be homogeneous by TLC. Unlabelled corticosterone was obtained from Roussel-Uclaf. The methods used in plasma preparation, measurement of corticosterone binding, determination of binding parameters and counting of radioactivity, were those previously described[3]. All data were analyzed by Student's t-test.

Results. The binding of corticosterone to plasma proteins of chick embryos was measured by equilibrium dialysis.

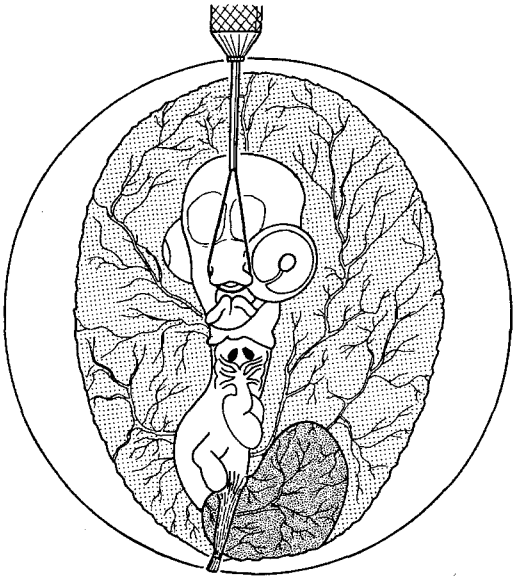

Figure 1. In this scheme the thyroid primordia are shown above the aortic arches; they are brought into view when the neck of the embryo is extended by means of a hairloop and of the amnion anchored onto the egg shell.

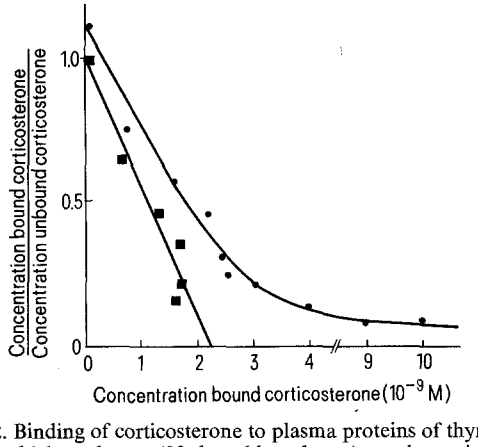

Figure 2. Binding of corticosterone to plasma proteins of thyroidectomized chick embryos (20-day-old embryos) as determined by equilibrium dialysis experiments with plasma diluted 1/28. Scatchard plot of total binding (●—●) and specific binding (Rosenthal's correction, □—□).

Figure 2 is a typical Scatchard plot for the interaction of thyroidectomized embryo plasma with corticosterone. The corticosterone binding isotherm showed the coexistence of 2 types of binding sites: 1 class of saturable and 1 class of unsaturable binding sites. The class of saturable interacting sites (i.e. transcortin) displayed a high affinity (K_A 4 °C: 0.5×10^9 M^{-1}). This K_A-value was not different from the mean K_A-value in normal embryos previously published (0.7×10^9 M^{-1}). The table presents the corticosterone binding capacity measured on plasma from normal and thyroidectomized embryos at different ages (days 13, 17 and 20). Since no sex difference in binding activity was apparent at any day of incubation[1] a single mean value was calculated for both sexes at each stage. At each stage, the mean values of corticosterone binding capacities of normal and thyroidectomized embryos were not significantly different. The rapid fall in transcortin concentration between days 17 and 20 was observed in both thyroidectomized embryos and normal embryos.

Discussion. Numerous investigations have been devoted to hypothyroid chick embryos; until now such embryos were always obtained through an indirect method: they were either hypophysectomized by partial decapitation[6,7], or treated with various antithyroid drugs[8]. As many different factors could be involved in those experiments, we have devised a new method for removing the thyroid gland prior to any sign of colloid synthesis. As thyroxine was found in the yolk of unincubated eggs[9], it is possible that our operated embryos do contain maternal thyroxine vertically transmitted. It is clear, that even if this is the case, a hypothyroid state is established at the end of incubation as testified by the failure of yolk sac retraction. Indeed withdrawal of the yolk sac has been shown to be linked with thyroid hormones[10] and no others.

Our results suggest that the hormonal control of transcortin cannot be correlated with the presence or the absence of thyroid in the chick embryo. The results extend beyond earlier observations[2] on the effect of thyroxine hormone on transcortin synthesis in vitro. Thyroxine had no effect upon the secretion of transcortin in culture medium by livers of 7-, 9- or 15-day-old chick embryos cultivated for 3 days. Ours results are in perfect agreement with those obtained by Labrie et al.[11] in humans; transcortin synthesis in humans is not influenced at all by thyroid hormones and remains normal in hyperthyroidism and hypothyroidism. On the contrary there are indications in the rat that thyroid hormones have a controlling influence on transcortin activity[4]. Thyroidectomy reduces, and administration of thyroid hormone enhances, transcortin activity in the rat[11]. From these latter indications, it seems that a unitary explanation of the transcortin regulating systems cannot be given.

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